ELSEVIER

Institut Pasteur

Microbes and Infection xx (2012) 1–9

www.elsevier.com/locate/micinf

Review

Mycobacterium tuberculosis modulators of the macrophage's cellular events

Valérie Poirier, Yossef Av-Gay*

Division of Infectious Diseases, Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada V6H 3Z6

Received 7 June 2012; accepted 4 July 2012

Abstract

A number of mycobacterial macromolecules have been shown to target biological processes within host macrophages; however, the exact mechanism for the majority of these host–pathogen interactions is poorly understood. The following review summarizes current knowledge and expands on a subset of mycobacterial effectors for which a cognate substrate, cellular partner or signaling pathway have been experimentally identified within the human host.

Keywords: Tuberculosis; *Mycobacterium tuberculosis*; Cell trafficking; Phagosome maturation; Antigen processing and presentation; Macrophages

1. Introduction

Mycobacterium tuberculosis (Mtb), the etiological agent of tuberculosis (TB), is one of the most prevalent infectious agents worldwide, infecting more than one billion people [1]. The World Health Organization estimated that 1.4 million people succumbed to the disease in 2010 (WHO Report, 2010). The combination of co-infection with HIV and the emergence of multi-drug resistant strains gives TB the highest mortality rate of any other infectious bacteria [1].

The focus of this review is to describe the strategies developed by Mtb to circumvent its destruction by interfering with host cellular pathways. While the list of Mtb effectors suggested to cross talk with the macrophage's killing machinery is growing, understanding of the mechanisms by which these effectors interact with host compounds is still very limited. In this paper, we will focus on Mtb macromolecules shown to interact directly with host protein or with a specific host pathway allowing Mtb to survive and replicate within the hostile environment of the macrophage.

2. Mycobacterial interference with macrophage cellular pathways

Interference with host cellular pathways is a common trait of bacterial pathogenesis. For instance, once internalized, *Listeria monocytogenes* evades phagosome–lysosome fusion and escapes the phagosome by secreting exotoxins LLO, PlcA and PlcB [2]. *Salmonella enterica*, on the other hand, activates acid tolerance genes allowing it to adapt to the low pH of the phagolysosome [3]. Lastly, *Legionella pneumophila* redirects phagosome maturation by altering maturation of the endocytic vesicle in which it resides. Rather than fusing with early endosomes, late endosomes and lysosomes, phagosomes containing *L. pneumophila* fuse with membranes derived from the reticulum endoplasmic creating a niche suited for bacterial replication [4].

Mtb secretes several proteins into standard culture media leading to the assumption that protein secretion would also occur in infected macrophages. Secreted proteins are thought to modulate host cell activities such as intracellular phagosome maturation and acidification [5]. Despite secreted proteins being the most studied Mtb proteins, little is known as to whether they are indeed secreted within the macrophage, and how they are directed from the bacterial cell into the phagosomal lumen and/or to the macrophage cytosol.

* Corresponding author. Jack Bell Research Centre, 2660 Oak Street, Vancouver BC, Canada V6H 3Z6. Tel.: +1 604 875 4329; fax: +1 888 224 7259.
E-mail address: yossi@mail.ubc.ca (Y. Av-Gay).

1286-4579/$ - see front matter © 2012 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.micinf.2012.07.001

Moreover, the unique cell wall structure of Mtb plays an important role in its pathogenicity [6]. Mtb cell wall components such as lipoarabinomannan (LAM), sulfolipids and trehalose dimycolate (Cord factor) are free lipids that have been shown to be important modulators of host immune processes [7]. Host immunity is therefore reduced [8] through the inhibition of interferon gamma (IFNγ)-mediated activation of macrophages [9], suppression of helper T cells [10], scavenging toxic oxygen intermediates [11] and inhibition of antigen presentation [12].

As Mtb pathogenicity and survival within the host relies on its effectors and their ability to interfere with the macrophage physiological pathways, we hypothesized that interference with host signaling pathways is one of the main mechanisms by which Mtb establishes successful infection [13].

2.1. Inhibition of phagosome maturation

Upon infection, macrophages engulf microorganisms and the killing and digestion process of the engulfed microbe is induced [13]. In order to achieve this killing, macrophages must undergo phagosome maturation; an essential process in which phagosomes interact with endosomes and lysosomes leading to the destruction of foreign particles. As illustrated in Fig. 1, phagosomes containing invading microorganisms normally interact with the endosomal pathway allowing for the exchange of endocytic solute materials and membrane components between the phagosomes and the endosomes [14]. These fusion events result in the acidification of the phagosomes through the acquisition of vesicular-H^+-ATPase pumps (V-ATPase) and proteolytic enzymes (lysosomal hydrolases). They also initiate the production of reactive oxygen intermediates and the processing of antigens for degradation of the organism [15].

The first line of defence against mycobacterial invasion are alveolar macrophages [13]. However, Mtb is able to circumvent the macrophage killing machinery. Mtb replication and persistence in macrophages relies on the inhibition of phagosome maturation and prevention of the phagolysosome fusion process (see Fig. 2). Armstrong and Hart [16] were the first ones to identify phagosome maturation arrest in macrophages infected with Mtb. They showed that approximately 70% of phagosomes containing Mtb did not fuse with lysosomes [16]. The blockage of phagosome maturation allows Mtb to avoid proteolytic degradation and antigen presentation required to initiate an adaptive immune response [16]. Phagosomes containing Mtb are therefore characterized by the absence of several phagosomal markers: the V-ATPase pump [15], the mannose receptor, lysosomal markers such as Lysosomal Associated Membrane Proteins (LAMP), hydrolases such as Cathepsin D [17,18], and the small GTPase Rab7 [19,20]. Several groups have shown that despite numerous studies displaying that Rab7 is not recruited to mycobacterial phagosomes [21,22], Rab7 can localize to these organelles [23,24]. Sun and colleagues reported Rab7 localization to *Mycobacterium bovis* BCG-containing phagosomes in Raw264.7 macrophages [23]. However, they noticed that Rab7 was found in its inactive GDP-form and hypothesized that once active Rab7 binds to the phagosomal membrane, it gets converted to the inactive GDP-form and remains attached to the membrane. Furthermore, inactive Rab7 cannot activate and recruit the lysosomal marker Rab-Interacting Lysosomal Protein (RILP) leading to prevention of phagolysosome fusion [23,25]. The absence of phagosomal markers in Mtb infected macrophages indicates that active interference with macrophage trafficking events occurs within the majority of phagosomes containing Mtb.

As a result of phagosome maturation arrest, Mtb infected macrophages undergo impaired antigen processing and

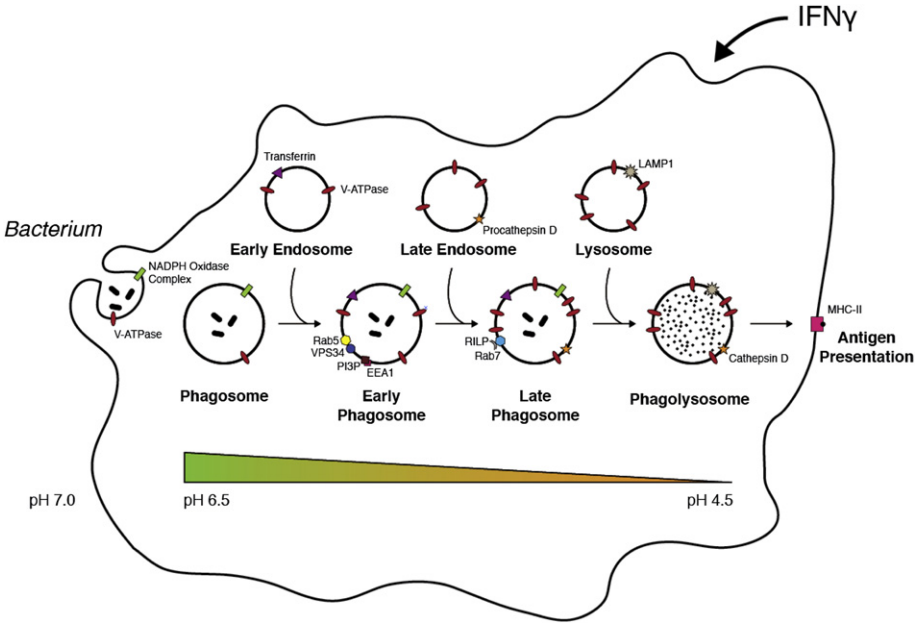

Fig. 1. Phagosome maturation engulfing an invader. As the phagosome matures, it undergoes multiple interactions with early endosomes, late endosomes, and lysosomes acquiring acidic and hydrolytic properties leading to the degradation of the pathogen.

Please cite this article in press as: V. Poirier, Y. Av-Gay, *Mycobacterium tuberculosis* modulators of the macrophage's cellular events, Microbes and Infection (2012), http://dx.doi.org/10.1016/j.micinf.2012.07.001

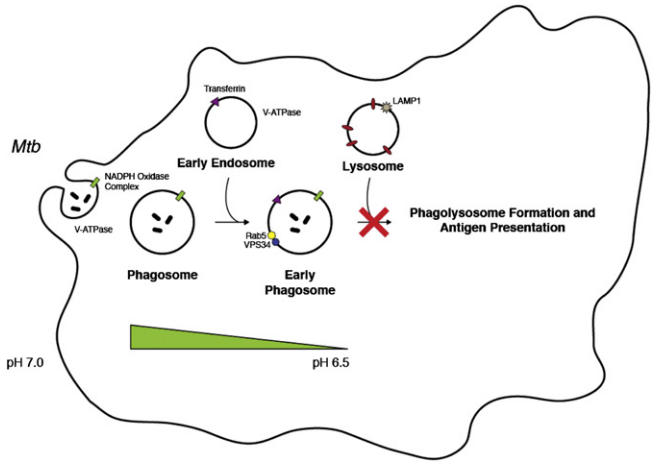

Fig. 2. Phagosome maturation when Mtb infects a macrophage. The fusion of the early endosome with the late endosome is inhibited by Mtb. The resulting phagosome has a relatively neutral pH and contains few hydrolytic enzymes required for the pathogen degradation.

presentation [12], reduced responsiveness to IFNγ [9], reduced production of cytokines, lower levels of reactive oxygen and nitrogen intermediates [11] and suppression of host cell apoptosis [26]. Mtb is able to target this biological function of the macrophage by utilizing an array of Mtb effectors composed of proteins and lipids.

2.1.1. LAM

Lipoarabinomannan (LAM), a lipoglycan, is a major component of Mtb cell wall. LAM includes three types of lipoglycans classified according to the presence and structure of the capping motif: mannosylated LAM (ManLAM), phosphoinositol-capped LAM (PiLAM) and arabinofuranosyl-terminated LAM (AraLAM) [27]. ManLAM is found on pathogenic mycobacterial species (e.g. *Mycobacterium tuberculosis*, *M. leprae* and *M. bovis*) whereas PiLAM and AraLAM are mainly found on non-pathogenic mycobacterial species [28]. For the purpose of this review, we will focus on ManLAM.

ManLAM is considered to be a key contributor to host-mycobacteria relationships by possessing immunomodulatory activities. Its main role is inhibition of calcium (Ca^{2+}) concentration rise in macrophages upon infection leading to interference with phagosome maturation [29]. Ca^{2+} serves as an essential cell signaling molecule, especially for the phagosome maturation process [29]. ManLAM was shown to specifically block the Ca^{2+} rise and to interfere with a sorting pathway that delivers lysosomal hydrolases and V-ATPases from the trans-Golgi network (TGN) to the phagosome [30]. ManLAM also inhibits the Ca^{2+}/calmodulin PI3K hVPS34 (human Vesicular Protein Sorting protein 34) pathways and blocks the syntaxin 6-dependent delivery of cargo from the TGN to the early endosome [31].

Upon phagocytosis of a pathogen, intracellular Ca^{2+} concentration normally rises leading to the recruitment of the early endosomal marker, the small GTPase Rab5, to the phagosomal membrane. Rab5 facilitates fusion events between early and late organelles of the endocytic pathway [21]. Rab5, along with calmodulin (CaM) and its effector Ca^{2+}/CaM kinase II (CaMKII), recruit type III PI3K hVPS34 to the phagosomal membrane [32]. PI3K VPS34 is essential for the production of the lipid regulator phosphatidylinositol 3-phosphate (PI3P) on the phagosomal membrane [33]. PI3P affects localization and function of proteins containing PI3P-binding domains (the FYVE zinc finger domain, the PH and PX domains). Proteins containing these domains are involved in membrane trafficking, endosomal protein sorting, multi-subunit enzyme assembly at the membrane and fusion [34]. One protein containing the FYVE domain is EEA1 (early endosome antigen 1) which is a tethering molecule and a Rab5 effector essential for fusion of early and late endosomes [31]. Its FYVE domain binds to PI3P causing membrane fusion by interacting with a family of proteins termed SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) composed of syntaxin 6, syntaxin 13 and additional elements such as the priming factor NSF and alpha-SNAP [35]. These interactions between PI3P and SNARE lead early endosomes to fuse with late endosomes allowing for the delivery of the endosomal cargo between them [36].

However, phagocytosis of Mtb results in a decrease in Ca^{2+} level in the macrophage [29] linked to the mycobacterial inhibition of CaMKII, a Ca^{2+} effector protein [37]. This inhibition hinders the recruitment of PI3K hVPS34 to the phagosomal membrane, the production of PI3P via the PI3K dependent pathway and the subsequent recruitment of EEA1 which drives fusion of early and late endosomes [30,32]. Although studies have shown that ManLAM is behind the block in intracellular Ca^{2+} rise leading to phagosome maturation arrest, it is still not known how ManLAM prevents intracellular Ca^{2+} levels from rising.

2.1.2. SapM

Prior to the discovery of SapM in Mtb, acid phosphatases were well recognized for their role in microbial pathogenicity [38,39]. Saleh et al. isolated a 28-kDa protein from Mtb culture filtrate and characterized it as the acid phosphatase SapM, for secreted acid phosphatase of *M. tuberculosis* [40]. SapM functions in an acidic-to-neutral pH range and uses organic phosphoesters as substrates [38]. The pH optimum for enzymatic rate of hydrolysis of the artificial protein tyrosine phosphatase substrate *p*NPP (*p*-nitrophenyl phosphate) was observed at pH 6.5–7.5 [40] by quantitation of released *p*NP (*p*-nitrophenol) [41] and P_i [42].

SapM was the first mycobacterial acid phosphatase to be identified and it appears to be the only secreted by Mtb. Interestingly, SapM is not found to have significant homology to prokaryotic acid phosphatases; rather, it shares sequence similarities with fungal acid phosphatases [40]. To date, there is minimal functional data on SapM homologues underlining the importance of performing site directed mutagenesis to determine the exact mode of action for SapM in Mtb.

Host substrates for SapM were identified by Vergne and colleagues. They observed that SapM could take active part in preventing Mtb phagosome maturation. They found that the

Please cite this article in press as: V. Poirier, Y. Av-Gay, *Mycobacterium tuberculosis* modulators of the macrophage's cellular events, Microbes and Infection (2012), http://dx.doi.org/10.1016/j.micinf.2012.07.001

exclusion of PI3P from phagosomes was only partly explained by the decrease in Ca^{2+} levels [22] and SapM was hydrolyzing the phagosomal membrane protein PI3P to PI [22]. PI3P serves as a membrane tag that signals phagosomes to mature down the phagolysosome biogenesis pathway [31]. They concluded that SapM dephosphorylation of PI3P prevented the recruitment of EEA1 to the phagosomal membrane and the fusion of phagosomes with late endosomes [22]. Vergne et al. also tested whether intracellular Ca^{2+} levels would restore PI3P levels on phagosomes containing live mycobacteria. They found that despite the Ca^{2+} rise, a transient spike in PI3P levels was shown to occur in only 10% of all phagosomes observed [22]. This suggests that reduced Ca^{2+} levels alone are not sufficient enough to explain the reduction of PI3P levels on mycobacterial phagosomes and reinforces the role of SapM in phagosome maturation arrest [22]. To accomplish complete arrest, Mtb must maintain a PI3P-free environment during its long-term stay in infected macrophages [22,29]. Thus, in addition to ManLAM, a second mycobacterial macromolecule, SapM, is required for complete inhibition of PI3P accumulation at the phagosomal membrane impeding EEA1 recruitment and fusion with late endosomes [22].

The story of SapM remains a conundrum as it is found to be secreted into the lumen of the phagosome but how it gains access to the cytoplasmic face of the phagosome where it hydrolyzes PI3P is unknown [22]. It is predicted that SapM contains an export signal allowing it to be exported to the cytosolic side of the phagosome. Nevertheless, SapM was experimentally proven to directly interfere with a host cellular pathway by hydrolyzing host PI3P into PI and resulting in phagosome maturation arrest [22,43].

2.1.3. PtpA

Studies from our laboratory showed that Mtb secretes a low molecular weight phosphatase, named protein tyrosine phosphatase A (PtpA), which is actively transcribed upon macrophage infection [44]. PtpA is essential for Mtb pathogenicity within macrophages where it was found to be secreted into the macrophage's cytosol. Once in the cytosol it interferes with the human macrophage signaling pathways by dephosphorylating a Vesicular Protein Sorting 33B (VPS33B) [43,45]. VPS33B is a key protein kinase ubiquitously expressed in eukaryotic cells that is involved in the regulation of vesicle trafficking and membrane fusion during phagocytosis and modulates the activity of SNAREs [46]. VPS33B dephosphorylation leads to the blockage of phagolysosome fusion [43]. VPS33B is recruited from the cytosol, and along with other vesicular sorting proteins (VPS11, VPS16 and VPS18), forms the class C complex [47]. The class C complex binds two additional proteins (VPS41 and VPS39) forming the HOPS (HOmotypic fusion and vacuole Protein Sorting) complex, a large multimeric tethering factor essential for vesicle fusion [48]. The HOPS complex is localized at the phagosomal membrane where it is required for phagosomal trafficking to the lysosome [49]. The HOPS complex is needed during the tethering and docking stages of vesicle fusion between the phagosomes and the lysosomes [50] where it plays a role in regulating the assembly of SNARE molecules through the interaction with endolysosomal Rabs [51]. Specifically, the HOPS complex associates with GTP-Rab7 through the interaction of VPS39 and VPS41 to promote tethering and SNARE-mediated membrane fusion of the phagolysosome [52]. These interactions permit the exchange of cytosolic contents and subsequent degradation of Mtb [53].

VPS39 has long been thought to be a guaninine-nucleotide exchange factor (GEF) of Rab7 exchanging GDP-Rab7 with GTP-Rab7 [54]. However, it was recently shown that VPS39 does not exhibit intrinsic nucleotide exchange activity towards Rab7 [55]. Genetic and biochemical evidences indicate that the complex Mon1-Ccz1 is the *bona fide* GEF of Rab7. Still, the interaction of VPS39 and VPS41 remains necessary for the binding of Rab7 to the HOPS complex [55].

Therefore, the HOPS complex is required for catalysts of docking, such as SNAREs and Rab7, to complete their roles in regulating the phagolysosome fusion and degradation of the pathogen [56]. However, as we have shown, Mtb PtpA interferes with this essential process by dephosphorylating VPS33B, thereby inhibiting the assembly of the HOPS complex, and preventing its interaction with Rab7 and assembly of SNAREs. Ultimately, dephosphorylation of VPS33B inhibits the fusion between the two organelles allowing Mtb to survive and grow within the enclosed and protected environment of the phagosome [43].

PtpA dephosphorylation of VPS33B is in concert with its binding to another host complex, the V-ATPase pump that controls phagosome acidification by transporting protons across membranes host protein [57]. During phagosome maturation, lysosomes extend along microtubules to fuse with and deliver the V-ATPase to the phagosome. Here, the proton pump recruits the HOPS complex for phagolysosome fusion [58] which generally results in a reduction in phagosomal pH from 6.5 to approximately 4.5 [59]. The binding of Mtb PtpA to the macrophage ATPase prevents co-localization of V-ATPase to the phagosome and blocks its acidification. PtpA binding to subunit H is essential for inhibiting V-ATPase trafficking to the mycobacterial phagosome during phagosome maturation. PtpA action prevents the recruitment of the HOPS complex by V-ATPase to the phagosome; an interaction essential for proper phagolysosome fusion and pathogen elimination [60]. The interaction of PtpA with subunit H of V-ATPase is a prerequisite for PtpA dephosphorylation of VPS33B. PtpA is responsible for disrupting the interaction between V-ATPase and the HOPS complex precluding the recruitment of the HOPS complex to the phagosome. Therefore, the inhibition of the phagolysosome fusion and phagosome maturation is dependent on both PtpA phosphatase activity (dephosphorylation of VPS33B) and its ability to bind to the host V-ATPase complex [60]. We now believe that the mechanism of action of PtpA is through first binding to the V-ATPase in the cytosol preventing its delivery to the phagosome. This brings PtpA to be located between the ATPase and in close proximity to the HOPS complex enabling the dephosphorylation of VPS33B.

Please cite this article in press as: V. Poirier, Y. Av-Gay, *Mycobacterium tuberculosis* modulators of the macrophage's cellular events, Microbes and Infection (2012), http://dx.doi.org/10.1016/j.micinf.2012.07.001

2.2. Inhibition of antigen processing and presentation

In addition to phagosome maturation arrest, to evade host immune response Mtb also modulates antigen processing and presentation. The major histocompatibility complex class II (MHC-II) is a receptor that presents digested bacterial antigenic peptides to helper T cells to initiate an adaptive immune response. Once the antigens are presented to helper T cells, the pro-inflammatory cytokine IFNɣ is secreted to activate infected macrophages by inducing a microbicidal response through the generation of reactive oxygen and nitrogen species, and by facilitating antigen processing and presentation [61]. IFNɣ plays a crucial role in activating an immune response and controlling macrophage infection by increasing the expression of MHC-II and accessory molecules required for antigen presentation [62]. Interestingly, upon binding to Toll-like receptor 2 (TLR2) located on macrophages, Mtb triggers a potent pro-inflammatory immune response early during infection. In later stages of infection, the pro-inflammatory response results in inhibition of antigen processing and presentation. It has been shown that inhibition of the pro-inflammatory response is required to combat the infection [63].

2.2.1. LpqH (19-kDa lipoprotein)

LpqH (19-kDa lipoprotein), classified as a pathogen-associated molecular pattern (PAMP) protein [63] is part of the Mtb cell wall [64]. There are currently no known functions of LpqH for Mtb cells grown *in vitro* and its biological activity is provided by its proposed role as an antigenic modulator of host immune processes [63]. LpqH binds to one of the macrophage Toll-Like Receptors, TLR2, to selectively inhibit IFNγ induction of the CIITA gene, the MHC-II gene and accessory protein genes required for antigen presentation [65,66]. CIITA, the MHC-II transactivator, regulates the expression of MCH-II and other genes involved in antigen processing and presentation by promoting the binding of transcription factors to the MHC-II promoter [66]. Upon binding to TLR2, it elicits a pro-inflammatory immune response in the host. This immune response helps combat the infection by inducing expression of cytokines, bacterial killing and apoptosis [67]. Interestingly, LpqH seems to have a pleiotropic effect on the immune system, i.e. its presence results in the activation of an immune response contributing to host defence, but also results in the inhibition of a subset of IFNγ responsive genes leading to persistence of the infection [68]. However, over a prolonged period some of these pro-inflammatory innate immune responses are down-regulated to limit damage to surrounding tissues [66].

The mechanism by which LpqH inhibits expression of MHC-II and antigen processing and presentation remains unclear. It is known, however, that the inhibition is dependent on TLR2 and MAPK signaling (p38 and/or ERK) [69] and that the expression of CIITA is suppressed [65,69]. Chromatin remodeling was suggested to be responsible for the inhibition of a subset of IFNγ-induced genes by Mtb; CIITA expression is dependent on changes in chromatin structure [66,69].

Eukaryotic DNA transcription is controlled by chromatin structure wrapped around histones. Unstructured histone tails can be targeted for post-translational modifications (e.g. phosphorylation, methylation, acetylation) to increase or decrease gene transcription. Histone acetylation is a transcription activator and it was observed that *MHC2TA*, the gene encoding CIITA, is silenced by deacetylation of histone in response to LpqH [70]. Pennini and colleagues showed that the CIITA promoter activity is inhibited by LpqH by a mechanism that involves inhibition of chromatin remodeling in a TLR2 and MAPK dependent manner [69]. They also showed that the IFNγ-induced acetylation of histones H3 and H4 was inhibited by LpqH. This suggests that the inhibition of chromatin remodeling and histone acetylation at the CIITA promoter accounts for the inhibition of CIITA mRNA expression [69]. Two models were developed by the same group in order to explain the inhibition of chromatin remodeling by LpqH. One model involves the reduced expression of histone acetyl transferase (HAT), known to catalyze acetylation of histones H3 and H4, and/or an increased expression of histone deacetylase (HDAC) that suppresses transcription through the deacetylation of histones. It was proposed that LpqH may inhibit the expression/activity of HAT required at the CIITA promoter or increase the expression/activity of HDAC that regulates the CIITA promoter [69]. The second model involves inhibiting the recruitment of certain transcription factors (e.g. Brahma-related gene 1 − Brg1) or the activation of CIITA repressors [69].

It is now known that LpqH inhibits CIITA expression via TLR2 and MAPK signaling. CIITA repression results in deacetylation of histones, a loss of chromatin remodeling and a decrease in subsequent expression of MHC-II and accessory molecules [69]. However, additional studies are required to confirm the exact mechanism of inhibition.

2.3. Hypothetical mycobacterium tuberculosis effectors

Mtb utilizes an arsenal of macromolecules to achieve inhibition of signaling pathways and to adapt to the hostile environment of the host. Those include a combination of cell wall compounds, proteins, glycolipids and glycopeptides which were shown to work in concert to promote Mtb survival within the macrophage. A list of these macromolecules is provided in Table 1. Several of these macromolecules have been shown to interfere directly with macrophage signaling pathways while others have been the focus of speculation and debate with their potential role in the interference of macrophage signaling. The implication of the following molecules in the macrophage remains to be determined.

The eukaryotic-like serine/threonine protein kinase PknG is a Mtb secreted protein that has been proposed to directly contribute to Mtb survival within the host. As claimed, PknG is believed to prevent lysosomal trafficking and phagosome maturation [71]. However, as the primary role of this protein kinase in Mtb is to regulate glutamine/glutamate metabolism, its contribution to phagosome maturation arrest may be an indirect effect [72] resulting from altered metabolism,

Please cite this article in press as: V. Poirier, Y. Av-Gay, *Mycobacterium tuberculosis* modulators of the macrophage's cellular events, Microbes and Infection (2012), http://dx.doi.org/10.1016/j.micinf.2012.07.001

Table 1
Mtb effectors involved in macrophage trafficking events and their substrates or targeted cellular pathways. (NA – not available).

Mtb effectors	Bacterial substrates/pathways targeted	Host substrates/pathways targeted
LAM	NA	Ca^{2+} concentration, Ca^{2+}/calmodulin PI3K hVPS34 pathway [29,32]
SapM	Organic phosphoesters [38]	PI3P [22]
PtpA	NA	Subunit H, VPS33B [43,60]
LpqH (19-kDa lipoprotein)	NA	Inhibits antigen processing and presentation [63]
PimB	LAM biosynthesis [73]	Involved in phagosome maturation via synthesis of LAM [82]
Trehalose dimycolate (Cord factor)	NA	Inhibits Ca^{2+}-induced fusion between phospholipid vesicles [75]
ESX-1 Component EspB (Rv3881c)	ESX-1 secretion apparatus [83]	No target identified/Involved in phagosome maturation arrest [74]
NdK	ATP for nucleoside diphosphate and triphosphate formation [76]	GTP-Rab5, GTP-Rab7 [23]
PknG	GarA [84]/Glutamine–glutamate metabolism [85]	Controversial [72]
LpdC	Pyruvate dehydrogenase complex [78]	Coronin-1/Phagosome maturation pathway [79]
MTSA-10	ESX-1 secretion apparatus [83]	Reduction of reactive oxygen species levels, activation of protein tyrosine phosphatases [80]

especially since no host proteins have been identified as targets or substrates of PknG.

Additional protein effectors have been suggested to play a role in the inhibition of phagosome maturation. These include PimB, a key mannosyltransferase that catalyzes the biosynthesis of LAM [73] and EspB, a protein secreted by the Mtb ESX-1 secretion system which is required for mycobacterial intracellular growth in macrophages [74]. Another example includes trehalose dimycolate (Cord factor), one of the major constituents of the Mtb cell wall, which is required for the inhibition of fusion of phospholipid vesicles and leads to the blockage of phagolysosome fusion. It achieves the blockage by inhibiting the Ca^{2+}-induced fusion between the phagosome and the lysosome phospholipid bilayers [75].

Another example of an Mtb protein shown to contribute to the inhibition of phagosome maturation is provided by Nucleotide diphosphate kinase (Ndk), an essential regulator of intracellular nucleotide pools allowing mycobacteria to maintain optimal physiological processes [76,77]. Ndk was found to be secreted within the host, bind macrophage GTP-Rab5 and GTP-Rab7, and hydrolyze them to inactive GDP forms leading to the inhibition of phagosome maturation [23].

Lipoamide dehydrogenase, LpdC, a member of Mtb's pyruvate dehydrogenase complex (PDH), also serves as the E1 compound of the peroxynitrite reductase/peroxidase (PNR/P) which helps Mtb resist host-reactive nitrogen intermediates [78]. Although its primary role is in Mtb physiology, LpdC was shown to retain coronin-1 at the phagosomal membrane correlating with phagosome maturation arrest [79] and to contribute to enhanced survival of Mtb within the macrophage. However, further investigations are required to clarify the mechanism behind this.

Lastly, Mtb Secreted Antigen (MTSA-10) binds to receptors on the macrophage surface and activates host cell protein tyrosine phosphatases in a redox-regulated fashion [80]. MTSA-10 was observed to downregulate reactive oxygen species levels via an unknown mechanism leading to activation of macrophage protein phosphatases. These then interfere with host cell signaling, downregulate transcription of genes essential for macrophage functions and prevent the macrophage from mounting an effective immune response [80,81].

3. Concluding remarks

This review addressed the Mtb effectors known to interfere with macrophage phagosome maturation and antigen processing and presentation. The effectors presented have defined host substrates or known targeted cellular pathways. These include LAM and SapM which work in parallel to keep Mtb in a PI3P-free environment and to inhibit the recruitment of EEA1 required for phagosome maturation, the secreted protein PtpA which disrupts the interaction between the HOPS complex and V-ATPase preventing acidification of the phagosome, and the cell wall component LpqH which inhibits antigen processing and presentation required for elimination of the pathogen.

As outlined above, an increasing number of Mtb effectors are suggested to interfere with macrophage's cellular response. Mtb seems to use multiple mechanisms in parallel to interfere with macrophage signaling pathways. The outcome of these actions is the establishment of an ideal niche within the phagosome where Mtb remains undetected by the immune system. Insight into the mode of action of these specific Mtb macromolecules is slowly emerging. However, the exact mechanisms by which these secreted molecules interfere with host signaling pathways remains under investigated and merit further scrutiny.

The identification of Mtb effectors interfering with macrophage trafficking and their mechanisms of action is important for our understanding of Mtb pathogenesis. Further characterization of these factors would allow us to understand the multiple pathways targeted by Mtb and would possibly open new avenues for pharmaceutical interventions.

Acknowledgements

We thank Stefan Szary for his help with graphical illustrations, and Jeffrey Helm and Melissa Richard-Greenblatt for

Please cite this article in press as: V. Poirier, Y. Av-Gay, *Mycobacterium tuberculosis* modulators of the macrophage's cellular events, Microbes and Infection (2012), http://dx.doi.org/10.1016/j.micinf.2012.07.001

proof reading of our manuscript. Funding for this research was provided by the Canadian Institute of Health Research operating grant # MOP-106622. We apologize to all investigators whose work could not be appropriately cited here due to space limitations.

References

[1] P.J. Dolin, M.C. Raviglione, A. Kochi, Global tuberculosis incidence and mortality during 1990–2000, W.H.O., Bull. 72 (1994) 213.
[2] R. Henry, L. Shaughnessy, M.J. Loessner, C. Alberti-Segui, D.E. Higgins, J.A. Swanson, Cytolysin-dependent delay of vacuole maturation in macrophages infected with *Listeria monocytogenes*, Cell Microbiol. 8 (2006) 107–119.
[3] L.R. Prost, M.E. Daley, V. Le Sage, M.W. Bader, H. Le Moual, R.E. Klevit, S.I. Miller, Activation of the bacterial sensor kinase PhoQ by acidic pH, Mol. Cell 26 (2007) 165–174.
[4] H. Brüggemann, C. Cazalet, C. Buchrieser, Adaptation of *Legionella pneumophila* to the host environment: role of protein secretion, effectors and eukaryotic-like proteins, Curr. Opin. Microbiol. 9 (2006) 86–94.
[5] D.G. Russell, Mycobacterium and Seduction of the Macrophage, Blackwell Science, Oxford, 1999.
[6] M. Daffé, P. Draper, The envelope layers of mycobacteria with reference to their pathogenicity, Adv. Microbiol. Physiol. 39 (1998) 131–203.
[7] W.L. Beatty, E.R. Rhoades, H.J. Ullrich, D. Chatterjee, J.E. Heuser, D.G. Russell, Trafficking and release of mycobacterial lipids from infected macrophages, Traffic 1 (2000) 235–247.
[8] P.J. Brennan, H. Nikaido, The envelope of mycobacteria, Annu. Rev. Biochem. 64 (1995) 29–63.
[9] L.D. Sibley, S.W. Hunter, P.J. Brennan, J.L. Krahenbuhl, Mycobacterial lipoarabinomannan inhibits gamma interferon-mediated activation of macrophages, Infect. Immun. 56 (1988) 1232–1236.
[10] J.J. Ellner, T.M. Daniel, Immunosuppression by mycobacterial arabinomannan, Clin. Exp. Immunol. 35 (1979) 250–257.
[11] J. Chan, X.D. Fan, S.W. Hunter, P.J. Brennan, B.R. Bloom, Lipoarabinomannan, a possible virulence factor involved in persistence of *Mycobacterium tuberculosis* within macrophages, Infect. Immun. 59 (1991) 1755–1761.
[12] C. Moreno, A. Mehlert, J. Lamb, The inhibitory effects of mycobacterial lipoarabinomannan and polysaccharides upon polyclonal and monoclonal human T cell proliferation, Clin. Exp. Immunol. 74 (1988) 206–210.
[13] A.L. Hestvik, Z. Hmama, Y. Av-Gay, Mycobacterial manipulation of the host cell, FEMS Microbiol. Rev. 29 (2005) 1041–1050.
[14] D.G. Russell, *Mycobacterium tuberculosis* and the intimate discourse of a chronic infection, Immunol. Rev. 240 (2011) 252–268.
[15] S. Sturgill-Koszycki, P.H. Schlesinger, P. Chakraborty, P.L. Haddix, H.L. Collins, A.K. Fok, R.D. Allen, S.L. Gluck, J. Heuser, D.G. Russell, Lack of acidification in mycobacterium phagosomes produced by exclusion of the vesicular proton-ATPase, Science 263 (1994) 678–681.
[16] J.A. Armstrong, P.D. Hart, Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes, J. Exp. Med. 134 (1971) 713–740.
[17] D.L. Clemens, M.A. Horwitz, Characterization of the *Mycobacterium tuberculosis* phagosome and evidence that phagosomal maturation is inhibited, J. Exp. Med. 181 (1995) 257–270.
[18] S. Sturgill-Koszycki, U.E. Schaible, D.G. Russell, Mycobacterium-containing phagosomes are accessible to early endosomes and reflect a transitional state in normal phagosome biogenesis, EMBO J. 15 (1996) 6960–6968.
[19] B. Hoflack, S. Kornfeld, Lysosomal enzyme binding to mouse P388D1 macrophage membranes lacking the 215-kDa mannose 6-phosphate receptor: evidence for the existence of a second mannose 6-phosphate receptor, Proc. Natl. Acad. Sci. 82 (1985) 4428–4432.
[20] S. Xu, A. Cooper, S. Sturgill-Koszycki, T. van Heyningen, D. Chatterjee, I. Orme, P. Allen, D.G. Russell, Intracellular trafficking in *Mycobacterium tuberculosis* and *Mycobacterium avium*-infected macrophages, J. Immunol. 153 (1994) 2568–2578.
[21] V. Derectic, L.E. Via, R.A. Fratti, D. Deretic, Mycobacterial phagosome maturation, Rab proteins, and intracellular trafficking, Electrophoresis 18 (1997) 2542–2547.
[22] I. Vergne, J. Chua, H.H. Lee, M. Lucas, J. Belisle, V. Deretic, Mechanism of phagolysosome biogenesis block by viable *Mycobacterium tuberculosis*, Proc. Natl. Acad. Sci. 102 (2005) 4033–4038.
[23] J. Sun, A.E. Deghmane, H. Soualhine, T. Hong, C. Bucci, A. Solodkin, Z. Hmama, *Mycobacterium bovis* BCG disrupts the interaction of Rab7 with RILP contributing to inhibition of phagosome maturation, J. Leukoc. Biol. 82 (2007) 1437–1445.
[24] S. Seto, K. Tsujimura, Y. Koide, Rab GTPases regulating phagosome maturation are differentially recruited to mycobacterial phagosomes, Traffic 12 (2011) 407–420.
[25] G. Cantalupo, P. Alifano, V. Roberti, C.B. Bruni, C. Bucci, Rab-interacting lysosomal protein (RILP): the Rab7 effector required for transport to lysosomes, EMBO J. 20 (2001) 683–693.
[26] M.K. Balcewicz-Sablinska, J. Keane, H. Kornfeld, H.G. Remold, Pathogenic *Mycobacterium tuberculosis* evades apoptosis of host macrophages by release of TNF-R2, resulting in inactivation of TNF-alpha, J. Immunol. 161 (1998) 2636–2641.
[27] D. Chatterjee, K.H. Khoo, Mycobacterial lipoarabinomannan: an extraordinary lipoheteroglycan with profound physiological effects, Glycobiology 8 (1998) 113–120.
[28] M. Gilleron, N. Himoudi, O. Adam, P. Constant, A. Venisse, M. Rivière, G. Puzo, *Mycobacterium smegmatis* phosphoinositols-glyceroarabinomannans: structure and localization of alkali-labile and alkali-stable phosphoinositides, J. Biol. Chem. 272 (1997) 117–124.
[29] Z.A. Malik, G.M. Denning, D.J. Kusner, Inhibition of Ca^{2+} signaling by *Mycobacterium tuberculosis* is associated with reduced phagosome–lysosome fusion and increased survival within human macrophages, J. Exp. Med. 191 (2000) 287–302.
[30] R.A. Fratti, J. Chua, I. Vergne, V. Deretic, *Mycobacterium tuberculosis* glycosylated phosphatidylinositol causes phagosome maturation arrest, Proc. Natl. Acad. Sci. 100 (2003) 5437–5442.
[31] R.A. Fratti, J.M. Backer, J. Gruenberg, S. Corvera, V. Deretic, Role of phosphatidylinositol 3-kinase and Rab5 effectors in phagosomal biogenesis and mycobacterial phagosome maturation arrest, J. Cell Biol. 154 (2001) 631–644.
[32] I. Vergne, J. Chua, V. Deretic, Tuberculosis toxin blocking phagosome maturation inhibits a novel Ca^{2+}/calmodulin-PI3K hVPS34 cascade, J. Exp. Med. 198 (2003) 653–659.
[33] S. Christoforidis, M. Miaczynska, K. Ashman, M. Wilm, L. Zhao, S.C. Yip, M.D. Waterfield, J.M. Backer, M. Zerial, Phosphatidylinositol-3-OH kinases are Rab5 effectors, Nat. Cell Biology 1 (1999) 249–252.
[34] M.A. Lemmon, Phosphoinositide recognition domains, Traffic 4 (2003) 201–213.
[35] H.M. McBride, V. Rybin, C. Murphy, A. Giner, R. Teasdale, M. Zerial, Oligomeric complexes link Rab5 effectors with NSF and drive membrane fusion via interactions between EEA1 and syntaxin 13, Cell 98 (1999) 377–386.
[36] A. Simonsen, J.M. Gaullier, A. D'Arrigo, H. Stenmark, The Rab5 effector EEA1 interacts directly with syntaxin-6, J. Biol. Chem. 274 (1999) 28857–28860.
[37] Z.A. Malik, C.R. Thompson, S. Hashimi, B. Porter, S.S. Lyer, D.J. Kusner, Cutting edge: *Mycobacterium tuberculosis* blocks Ca^{2+} signaling and phagosome maturation in human macrophages via specific inhibition of sphingosine kinase, J. Immunol. 170 (2003) 2811–2815.
[38] G.M. Rossolini, S. Schippa, M.L. Riccio, F. Berlutti, L.E. Macaskie, M.C. Thaller, Bacterial nonspecific acid phosphohydrolases: physiology, evolution and use as tools in microbial biotechnology, Cell Mol. Life Sci. 54 (1998) 833–850.
[39] A.T. Remaley, S. Das, P.I. Campbell, G.M. Larocca, M.T. Pope, R.H. Glew, Characterization of *Leishmania donovani* acid phosphatases, J. Biol. Chem. 260 (1985) 880–886.
[40] M.T. Saleh, J.T. Belisle, Secretion of an acid phosphatase (SapM) by *Mycobacterium tuberculosis* that is similar to eukaryotic acid phosphatases, J. Bacteriol. 182 (2000) 6850–6853.

[41] G.N. Bowers, R.B. McComb, A. Upretti, 4-nitrophenyl phosphate characterization of high purity materials for measuring alkaline phosphatase activity in human serum, Clin. Chem. 27 (1981) 135–143.
[42] J. Murphy, J.P. Riley, A modified single method for the determination of phosphate in neutral waters, Anal. Chim. Acta 27 (1962) 31–36.
[43] H. Bach, K.G. Papavinasasundaram, D. Wong, Z. Hmama, Y. Av-Gay, *Mycobacterium tuberculosis* virulence is mediated by PtpA dephosphorylation of human vacuolar protein sorting 33B, Cell Host Microbe 3 (2008) 316–322.
[44] S.C. Cowley, R. Babakaiff, Y. Av-Gay, Expression and localization of the *Mycobacterium tuberculosis* protein tyrosine phosphatase PtpA, Res. Microbiol. 153 (2002) 233–241.
[45] H. Bach, J. Sun, Z. Hmama, Y. Av-Gay, *Mycobacterium avium* subsp. paratuberculosis PtpA is an endogenous tyrosine phosphatase secreted during infection, Infect. Immun. 74 (2006) 6540–6546.
[46] M. Huizing, A. Didier, J. Walenta, Y. Anikster, W.A. Gahl, H. Kramer, Molecular cloning and characterization of human VPS18, VPS 11, VPS16, and VPS33, Gene 264 (2001) 241–247.
[47] L.M. Banta, J.S. Robinson, D.J. Klionsky, S.D. Emr, Organelle assembly in yeast: characterization of yeast mutants defective in vacuolar biogenesis and protein sorting, J. Cell Biol. 107 (1988) 1369–1383.
[48] T. Darsow, S.E. Reider, S.D. Emr, A multispecificity syntaxin homologue, Vam3p, essential for autophagic and biosynthetic protein transport to the vacuole, J. Cell Biol. 138 (1997) 517–529.
[49] T. Vida, B. Gerhardt, A cell-free assay allows reconstitution of Vps33p-dependent transport to the yeast vacuole/lysosome, J. Cell Biol. 146 (1999) 85–97.
[50] A. Price, W. Wickner, C. Ungermann, Proteins needed for vesicle budding from the golgi complex are also required for the docking step of homotypic vacuole fusion, J. Cell Biol. 148 (2000b) 1223–1229.
[51] L.M. Banta, T.A. Vida, P.K. Herman, S.D. Emr, Characterization of yeast Vps33p, a protein required for vacuolar protein sorting and vacuole biogenesis, Mol. Cell Biol. 10 (1990) 4638–4649.
[52] T.K. Sato, P. Rehling, M.R. Peterson, S.D. Emr, C. Class, Vps protein complex regulates vacuolar SNARE pairing and is required for vesicle docking/fusion, Mol. Cell 6 (2000) 661–671.
[53] D.F. Seals, G. Eitzen, N. Margolis, W.T. Wickner, A. Price, A Ypt/Rab effector complex containing the Sec1 homolog Vps33p is required for homotypic vacuole fusion, Proc. Natl. Acad. Sci. 97 (2000) 9402–9407.
[54] A.E. Wurmser, T.K. Sato, S.D. Emr, New component of the vacuolar class C-Vps complex couples nucleotide exchange on the Ypt7 GTPase to SNARE-dependent docking and fusion, J. Cell Biol. 151 (2000) 551–562.
[55] M. Nordmann, M. Cabrera, A. Perz, C. Brocker, C. Ostrowicz, S. Engelbrecht-Vandre, C. Ungermann, The Mon1-Ccz1 complex is the GEF of the late endosomal Rab7 homolog Ypt7, Curr. Biol. 20 (2010) 1654–1659.
[56] C. Stroupe, K.M. Collins, R.A. Fratti, W. Wickner, Purification of active HOPS complex reveals its affinities for phosphoinositides and the SNARE Vam7p, EMBO J. 25 (2006) 1579–1589.
[57] D.J. Hackam, O.D. Rotstein, W.J. Zhang, N. Demaurex, M. Woodside, O. Tsai, S. Grinstein, Regulation of phagosomal acidification. Differential targeting of Na+/H+ exchangers, Na+/K+-ATPases, and vacuolar-type H+-ATPases, J. Biol. Chem. 272 (1997) 29810–29820.
[58] G.H. Sun-Wada, H. Tabata, N. Kawamura, M. Aoyama, Y. Wada, Direct recruitment of H+-ATPase from lysosomes for phagosomal acidification, J. Cell Sci. 122 (2009) 2504–2513.
[59] S. Mukherjee, R.N. Ghosh, F.R. Maxfield, Endocytosis, Physiol. Rev. 77 (1997) 759–803.
[60] D. Wong, H. Bach, Z. Hmama, Y. Av-Gay, *Mycobacterium tuberculosis* protein tyrosine phosphatase A disrupts phagosome acidification by exclusion of host vacuolar-H+-ATPase, Proc. Natl. Acad. Sci. 108 (2011) 19371–19376.
[61] D.G. Russell, Who puts the tubercle in tuberculosis? Nat. Rev. Microbiol. 5 (2007) 39–47.
[62] P.P. Singh, C. LeMaire, J.C. Tan, E. Zeng, J.S. Schorey, Exosomes released from *M. tuberculosis* infected cells can suppress IFN-γ mediated activation of naïve macrophages, PLoS ONE 6 (2011) e18564.
[63] E.H. Noss, R.K. Pai, T.J. Sellati, J.D. Radolf, J. Belisle, D.T. Golenbock, W.H. Boom, C.V. Harding, Toll-like receptor 2-dependent inhibition of macrophage class II MHC expression and antigen processing by 19-kDa lipoprotein of *Mycobacterium tuberculosis*, J. Immunol. 167 (2001) 910–918.
[64] D.B. Young, T. Garbe, Lipoprotein antigens of *M. tuberculosis*, Res. Microbiol. 142 (1991) 55–65.
[65] R.K. Pai, M. Convery, T.A. Hamilton, W.H. Boom, C.V. Harding, Inhibition of IFN-γ-induced class II transactivator expression by a 19-kDa lipoprotein from *Mycobacterium tuberculosis*: a potential mechanism for immune evasion, J. Immunol. 171 (2003) 175–184.
[66] R.K. Pai, M.E. Pennini, A.A. Tobian, D.H. Canaday, W.H. Boom, C.V. Harding, Prolonged toll-like receptor signaling by *Mycobacterium tuberculosis* and its 19-kilodalton lipoprotein inhibits gamma interferon-induced regulation of selected genes in macrophages, Infect. Immun. 72 (2004) 6603–6614.
[67] H.D. Brightbill, D.H. Libraty, S.R. Krutzik, R.B. Yang, J.T. Belisle, J.R. Bleharski, M. Maitland, M.V. Norgard, S.E. Plevy, S.T. Smale, P.J. Brennan, B.R. Bloom, P.J. Godowski, R.L. Modlin, Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors, Science 285 (1999) 732–736.
[68] R.S. Chu, D. Askew, E.H. Noss, A. Tobian, A.M. Krieg, C.V. Harding, CpG oligodeoxynucleotides down-regulate macrophage class II MHC antigen processing, J. Immunol. 163 (1999) 1188.
[69] M.E. Pennini, R.K. Pai, D.C. Schultz, W.H. Boom, C.V. Harding, *Mycobacterium tuberculosis* 19-kDa lipoprotein inhibits IFN-gamma-induced chromatin remodeling of MHC2TA by TLR2 and MAPK signalling, J. Immunol. 176 (2006) 4323–4330.
[70] S. Landmann, A. Mühlethaler-Mottet, L. Bernasconi, T. Suter, J.M. Waldburger, K. Masternak, J.F. Arrighi, C. Hauser, A. Fontana, W. Reith, Maturation of dendritic cells is accompanied by rapid transcriptional silencing of class II transactivator (CIITA) expression, J. Exp. Med. 194 (2001) 379–392.
[71] A. Walburger, A. Koul, G. Ferrari, L. Nguyen, C. Prescianotto-Baschong, K. Huygen, B. Klebl, C. Thompson, G. Bacher, J. Pieters, Protein kinase G from pathogenic mycobacteria promotes survival within macrophages, Science 304 (2004) 1800–1804.
[72] J. Chao, D. Wong, X. Zheng, V. Poirier, H. Bach, Z. Hmama, Y. Av-Gay, Protein kinase and phosphatase signaling in *Mycobacterium tuberculosis* physiology and pathogenesis, Biochim. Biophys. Acta 1804 (2009) 620–627.
[73] M.L. Schaeffer, K.H. Khoo, G.S. Besra, D. Chatterjee, P.J. Brennan, J.T. Belisle, J.M. Inamine, The PimB gene of *Mycobacterium tuberculosis* encodes a mannosyltransferase involved in lipoarabinomannan biosynthesis, J. Biol. Chem. 274 (1999) 31625–31631.
[74] J. Xu, O. Laine, M. Masciocchi, J. Manoranjan, J. Smith, S.J. Du, N. Edwards, X. Zhu, C. Fenselau, L.Y. Gao, A unique mycobacterium ESX-1 protein co-secretes with CFP-10/ESAT-6 and is necessary for inhibiting phagosome maturation, Mol. Microbiol. 66 (2007).
[75] B.J. Spargo, L.M. Crowe, T. Ioneda, B.L. Beaman, J.H. Crowe, Cord factor (alpha, alpha-trehalose 6,6'-dimycolate) inhibits fusion between phospholipid vesicles, Proc. Natl. Acad. Sci. 88 (1991) 737–740.
[76] A.M. Chakrabarty, Nucleoside diphosphate kinase: role in bacterial growth, virulence, cell signalling and polysaccharide synthesis, Mol. Microbiol. 28 (1998) 875–882.
[77] I. Lascu, P. Gonin, The catalytic mechanism of nucleoside diphosphate kinases, J. Bioenerg. Biomembr. 32 (2000) 237–246.
[78] A. Venugopal, R. Bryk, S. Shi, K. Rhee, P. Rath, D. Schnappinger, S. Ehrt, C. Nathan, Virulence of *Mycobacterium tuberculosis* depends on lipoamide dehydrogenase, a member of three multienzyme complexes, Cell Host Microbe 9 (2011) 21–31.
[79] A.E. Deghmane, H. Soualhine, H. Bach, K. Sendide, S. Itoh, A. Tam, S. Noubir, A. Talal, R. Lo, S. Toyoshima, Y. Av-Gay, Z. Hmama, Lipoamide dehydrogenase mediates retention of coronin-1 on BCG vacuoles, leading to arrest in phagosome maturation, J. Cell Sci. 120 (2007) 2796–2806.
[80] S.K. Basu, D. Kumar, D.K. Singh, N. Ganguly, Z. Siddiqui, K.V. Rao, P. Sharma, *Mycobacterium tuberculosis* secreted antigen (MTSA-10)

Please cite this article in press as: V. Poirier, Y. Av-Gay, *Mycobacterium tuberculosis* modulators of the macrophage's cellular events, Microbes and Infection (2012), http://dx.doi.org/10.1016/j.micinf.2012.07.001

modulates macrophage function by redox regulation of phosphatases, FEBS J. 273 (2006) 5517−5534.

[81] S.K. Basu, D. Kumar, N. Ganguly, K.V. Rao, P. Sharma, *Mycobacterium tuberculosis* secreted antigen (MTSA-10) inhibits macrophage response to lipopolysaccharide by redox regulation of phosphatases, Indian J. Exp. Biol. 47 (2009) 505−519.

[82] J.B. Torrelles, L.E. Desjardins, J. MacNeil, T.M. Kaufman, B. Kutzbach, R. Knaup, T.R. McCarthy, S.S. Gurcha, G.S. Besra, S. Clegg, L.S. Schlesinger, Inactivation of Mycobacterium tuberculosis mannosyltransferase pimB reduces the cell wall lipoarabinomannan and lipomannan content and increases the rate of bacterial-induced human macrophage cell death, Glycobiology 19 (2009) 743−755.

[83] B. McLaughlin, J.S. Chon, J.A. MacGurn, F. Carlsson, T.L. Cheng, J.S. Cox, E.J. Brown, A mycobacterium ESX-1-secreted virulence factor with unique requirements for export, PLoS ONE 3 (2007) e105.

[84] H.M. O'Hare, R. Duran, C. Cervenansky, M. Bellinzoni, A.M. Wehenkel, O. Pritsch, G. Obal, J. Baumgartner, J. Vialaret, K. Johnsson, P.M. Alzari, Regulation of glutamate metabolism by protein kinases in mycobacteria, Mol. Microbiol. 70 (2008) 1408−1423.

[85] S. Cowley, M. Ko, N. Pick, R. Chow, K.J. Downing, B.G. Gordhan, J.C. Betts, V. Mizrahi, D.A. Smith, R.W. Stokes, Y. Av-Gay, The *Mycobacterium tuberculosis* protein serine/threonine kinase PknG is linked to cellular glutamate/glutamine levels and is important for growth *in vivo*, Mol. Microbiol. 52 (2004) 1691−1702.

Please cite this article in press as: V. Poirier, Y. Av-Gay, *Mycobacterium tuberculosis* modulators of the macrophage's cellular events, Microbes and Infection (2012), http://dx.doi.org/10.1016/j.micinf.2012.07.001